APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1992, p. 3047–3052 Vol. 58, No. 9
0099-2240/92/093047-06$02.00/0

Assessment of *Pediococcus acidilactici* as a Potential Silage Inoculant

A. FITZSIMONS,[1]* F. DUFFNER,[1] D. CURTIN,[1] G. BROPHY,[1] P. O'KIELY,[2] AND M. O'CONNELL[1]

School of Biological Sciences, Dublin City University, Glasnevin, Dublin 9,[1] and Teagasc, Grange Research Centre, Dunsany, County Meath,[2] Ireland

Received 2 December 1991/Accepted 4 July 1992

Eighteen *Pediococcus* strains were screened for their potential as silage inoculants. *Pediococcus acidilactici* G24 was found to be the most suitable, exhibiting a short lag phase on both glucose and fructose, a rapid rate of acid production, a high sugar-to-lactate conversion efficiency, no detectable breakdown of proteins or lactic acid, and the ability to grow within a broad range of pH and temperature. When tested in laboratory silos using grass with a water-soluble carbohydrate content of 24 g/kg of aqueous extract, *P. acidilactici* G24 stimulated the natural *Lactobacillus plantarum* population and accelerated the rates of lactic acid production and pH decrease. After 6 days of fermentation, the inoculated silage exhibited a 12% decrease in ammonia nitrogen and an 11% increase in crude protein levels compared with uninoculated controls. The use of an *L. plantarum* inoculant at a rate of 10^4 bacteria per g of grass in conjunction with *P. acidilactici* G24 produced no additional beneficial effect. Inoculation of grass with a water-soluble carbohydrate level of 8 g/kg of aqueous extract with *P. acidilactici* G24 led to no acceleration in the rate of *L. plantarum* growth or pH decrease. However, after 7 days of fermentation the inoculated silage had a 14% lower ammonia nitrogen protein content than did uninoculated controls. The results suggest that *P. acidilactici* G24 may be useful as a silage inoculant for crops with a sufficiently high water-soluble carbohydrate level.

Silage is defined as the product formed when grass or other material of sufficiently high moisture content, liable to spoilage by aerobic microorganisms, is stored anaerobically (25). The objective of silage making is efficient preservation of the crop, so that losses in nutritional value are minimized. After cutting, the crop is placed in a silo and sealed from the atmosphere. Respiratory activity by plant enzymes and aerobic microorganisms results in a rapid decrease in oxygen levels (6). Once anaerobic conditions are established, lactic acid bacteria present on the crop prior to harvesting rapidly multiply and ferment crop water-soluble carbohydrates to lactic and acetic acids. The low pH and the toxicity of the undissociated acids restrict further microbial activity and facilitate preservation of the remaining crop nutrients (17). If a low pH is not rapidly established, a secondary fermentation can occur in which clostridia ferment lactic acid and crop water-soluble carbohydrates to butyric acid and carbon dioxide. In this process, 2 mol of lactic acid is converted into 1 mol of the weaker butyric acid and an increase in pH takes place. Conditions then become favorable for the growth of proteolytic clostridia, which convert crop proteins and amino acids to ammoniacal compounds, thus further increasing the pH and degrading crop nutrients in the process (25). Crop preservation is therefore dependent on the rapid onset of lactic acid fermentation, bringing about a sharp decrease in pH. While numbers of lactic acid bacteria on fresh grass vary from 10^3 to 10^7/g of herbage (11, 14, 15), homofermentative acid-tolerant lactic acid bacteria, which are highly efficient in lactate production, constitute a small fraction of the total population (4). Inoculation of silage with large quantities of homofermentative lactic acid bacteria has therefore been proposed as a means of aiding preservation. For this strategy to be successful, the inoculant must satisfy certain criteria, as outlined by Whittenbury (22). These include rapid growth and lactate production, leading to a sharp decrease in pH; the ability to ferment the major crop carbohydrates, glucose and fructose, to lactate in a homofermentative fashion; tolerance of environmental conditions of temperature and pH in the silo; and absence of proteolytic activity. Of the lactic acid bacteria, only the pediococci and the homofermentative lactobacilli satisfy these criteria (2). Owing to the difficulty of isolating a single strain that meets all of these criteria, a mixed culture appears to have greater potential as an effective inoculant. The use of *Lactobacillus plantarum* strains only in silage inoculants is uncommon, since grass at ensiling has a pH of approximately 6 while *L. plantarum* is slow to produce lactic acid until a pH of less than 5 is reached (10). A number of researchers have found that pediococci dominate the early stages of ensilage (7, 9, 23) and have proposed that a mixed culture of pediococci, which would initiate fermentation and rapidly decrease the pH to 5, and *L. plantarum*, which would then further decrease the pH, might prove effective as a silage inoculant. Experimental studies support this theory, with mixed inocula of *L. plantarum* and *Pediococcus acidilactici* proving particularly effective (3, 5, 21). These findings are reflected in the composition of the 16 inoculants currently available on the Irish market, of which 13 contain both *L. plantarum* and *Pediococcus* strains, 2 contain only *L. plantarum*, and one contains a single *Pediococcus* strain (20). While considerable data on the evaluation of *L. plantarum* strains as silage inoculants are available relatively few publications have focussed on the selection of *Pediococcus* strains, with most *Pediococcus*-related publications dealing with preformulated mixed-inoculum performance. This is unfortunate considering the well-documented role of pediococci in silage fermentation. The object of the experiments reported here was evaluation of a number of *P. acidilactici* strains as potential silage inoculants.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. *Bacillus cereus* was obtained from laboratory stocks. *P. acidilactici* PLL01, PLL02, PLL03, PLL04, PLL05, PLL06, PLL07, PLL08, and PLL09 and *L. plantarum* L115 were obtained from V. Laffitte, Lacto Labo, Dange-Saint-Romain, France. *P. acidilactici* M408, P17, E92, E112, and E165 were supplied by F. Dellaglio, Universita Cattolica del Sacro Cuore, Piacenza, Italy. *P. acidilactici* A12, B14, C20, and G24 were isolated from different grass silages on the basis of the ability to grow at 50°C and to ferment D-arabinose to acid (2). The isolation procedure involved addition of a 10-g silage sample to 90 ml of sterile Ringer's solution and homogenization. The silage extract was diluted in 10-fold steps to 10^{-4}, and 0.1 ml of each dilution was spread on MRS (Oxoid) medium containing 2 μg of amphotericin B (GIBCO) per ml. Following overnight incubation at 50°C, colonies were transferred onto MRS plates containing 20 g of D-arabinose per liter as the sole carbon source and 20 ml of a 30-g/liter solution of bromocresol green per liter in 0.01 N NaOH. Colonies capable of fermenting D-arabinose appeared yellow after overnight incubation at 37°C and were subjected to microscopic examination. All gram-positive cocci were tested for the ability to ferment glucose, fructose, galactose, arabinose, xylose, and maltose to acid to confirm their identity. Glucose fermentation was assayed in MRS broth containing 0.0016% bromocresol green as an indicator. Other fermentation tests required replacement of glucose with the appropriate sugar. Sugar utilization was indicated by a change in the medium from green to yellow due to acid production.

Growth conditions. Pediococci and lactobacilli were cultured on MRS (Oxoid) at 37°C, except as otherwise stated.

Growth characteristics. The design of methods for determining growth characteristics was based on the characteristics required of a successful silage inoculant. All growth characterizations (growth curves, kinetic studies, sugar-to-lactate conversions, etc.) were done with MRS as the growth medium.

Optimal growth temperatures were determined by inoculation of culture medium preincubated at selected temperatures from 15 to 45°C, followed by incubation for 24 h at the desired temperature. The effect of pH values between 4 and 8 on growth was assessed by inoculation of culture medium which had been adjusted to the desired pH by using concentrated HCl-H_2SO_4 and subsequent incubation at 37°C for 12 h. A 0.1% (vol/vol) inoculum from a static culture was used in all cases, and culture turbidity at 600 nm was used to monitor cell growth. Optical densities were plotted against pH and temperature, and the optimum values of pH and incubation temperature for growth were determined graphically. The percentage of maximum growth attained after 12 h at 20°C was determined by simultaneously inoculating MRS broth preheated to both 20 and 37°C (the temperature optimum for *P. acidilactici*). Following incubation for 12 h at these temperatures, optical densities were compared.

Growth curves were determined by using 100 ml of MRS containing glucose or fructose as the sole carbon source which was inoculated from a static culture at a rate of 10^6 cells per ml. Cell numbers were monitored by sampling and dilution in Ringer's solution, followed by plating on MRS and overnight incubation at 37°C. Growth curves were determined at 30°C, as opposed to the optimum growth temperature of 37°C, to approximate silo conditions more closely. Exponential growth rates were calculated from the bacterial concentrations X_0 and X_t at times t_0 and t with the equation $\mu = (\log X_t - \log X_0)/\log e\ (t - t_0)$, where $\log e = 0.43429$ (16).

The lag phase was defined as the time between inoculation and establishment of the maximum growth rate (16) and was determined graphically by using a plot of log cell number versus time.

Cultures used for growth curves were sampled after 12 h for determination of sugar-to-lactate conversion efficiencies and were also used to measure culture pH after 26 h of growth. Glucose-to-lactate conversion efficiencies were calculated on the basis of the fact that fermentation of 1 mol of glucose to 2 mol of lactate represents 100% conversion. Sampling at such an early stage in the growth cycle led to conversion efficiencies lower than those that might be expected for a homofermentative organism such as *P. acidilactici*. However, this was deemed necessary in view of the importance of the early stages of silage fermentation in determining the success of preservation.

Inhibition of *L. plantarum*. Test strains were dotted onto MRS agar by using sterile toothpicks and incubated overnight at 37°C. Colonies were lysed by exposure to chloroform for 30 min and overlaid with soft MRS agar seeded with 10^6 *L. plantarum* cells per ml. Once solid, plates were incubated overnight at 37°C and screened for zones of inhibition.

Proteolytic activity. Cells were streaked on MRS medium containing 5% gelatin as the sole carbon source. After 48 h of incubation at 30°C, plates were flooded with a solution of 15 g of mercuric chloride in 20 ml of concentrated HCl and 100 ml of distilled water. *B. cereus* was used as a positive control, and proteolysis was detected as a clear zone surrounding areas of bacterial growth against a hazy white background.

Biochemical assays. Reducing sugar concentrations were measured by using 5-dinitrosalicylic acid and the method of Bernfeld (1). Lactic acid levels in culture broths and silage extracts were determined enzymatically by using beef heart L-(+)-lactic dehydrogenase and *L. leichmannii* D-(−)-lactic dehydrogenase (Boehringer Mannheim Corp.).

Laboratory scale silos. Cylindrical silos with a 6-kg capacity, as described by O'Kiely and Wilson (13), were used in all experiments. Inocula were grown to the stationary phase in MRS at 37°C. Plate counts on MRS from cultures grown previously with identical initial cell concentrations MRS batches, and incubation temperatures and times were used to estimate inoculant cell density. The culture volume required to treat 7 kg of grass at the desired rate of bacteria per g of grass (2 to 4 ml) was diluted to 10 ml in sterile Ringer's solution. Seven kilograms of perennial ryegrass (*Lolium perenne*) chopped by a precision chop harvester was spread on a sterile plastic sheet, and half of the inoculum was added dropwise by using a syringe. The grass was hand mixed, and the remaining inoculum was added. It was assumed that small amounts of MRS which had supported bacterial growth to the stationary phase would have no effect on the silage fermentation, and uninoculated controls were therefore treated with 10 ml of sterile Ringer's solution. Following inoculation, 6 kg of treated grass was packed into a test silo which was immediately sealed. For each treatment, sufficient silos were set up to allow three silos to be opened on each day on which sampling was required. Silos were sampled by emptying and thoroughly mixing the entire contents of each silo and then removing a sample of approximately 100 g.

Microbiological analysis of silage. A 10-g sample of silage was added to 90 ml of sterile Ringer's solution and homog-

enized. An extract was diluted in 10-fold steps in sterile Ringer's solution, and the appropriate dilutions were plated. *L. plantarum* numbers were estimated by plating on LP agar, a selective medium based on the growth characteristics of lactobacilli as listed by Sharpe (19) and described below and the sugar fermentation profile of *L. plantarum*. The medium was prepared by dissolving 10 g of peptone, 5 g of tryptone, 5 g of yeast extract, 6 g of KH_2PO_4, 2.5 g of sodium acetate, 2 g of diammonium citrate, 1 ml of Tween 80, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $MnSO_4 \cdot 4H_2O$, and 20 g of sorbitol in 900 ml of distilled water. Following addition of 20 ml of indicator solution containing 0.1 g of bromocresol green in 30 ml of 0.01 N NaOH, the pH was brought to 5.5 by using glacial acetic acid. Agar was added to 1.2%, and the total volume was brought to 1 liter with distilled water. The medium was autoclaved at 110°C for 20 min, and on cooling to 55°C, 2 μg of amphotericin B per ml was added to prevent fungal growth. Plates were incubated for 48 h at 30°C. *L. plantarum* colonies appeared large and yellow against a background of small green-blue colonies. The low pH, the high concentration of acetate ions, and the presence of the growth stimulant Tween 80 in LP medium facilitate selection of lactobacilli (19). Use of sorbitol as the sole sugar source ensures that of the lactobacilli only *L. plantarum*, *L. salivarius*, *L. casei*, and some strains of *L. coryneformis* produce acid (2) and form large yellow colonies. Of these four species, only *L. plantarum* ferments both ribose and raffinose (2), and 500 yellow colonies isolated in this manner from each treatment were replica plated on LP medium containing each of these sugars as the sole carbon source. The percentage of yellow colonies positively identified as *L. plantarum* varied from 97 to 98% in samples from silos which had not been inoculated with *L. plantarum* to 99 to 100% in samples from treated silos. In each treatment, the number of yellow colonies was multiplied by the relevant conversion rate to determine the actual number of *L. plantarum* colonies.

No such selective medium based on sugar fermentation profiles could be developed for *P. acidilactici*. However, *P. acidilactici* is one of the few silage microorganisms capable of growth at temperatures as high as 50°C (2). Samples were therefore plated on MRS containing 2μg of amphotericin B per ml to inhibit fungal growth and incubated overnight at 50°C. *P. acidilactici* ferments glucose, fructose, galactose, arabinose, and xylose but not maltose to acid, and 500 colonies isolated in this manner from each treatment were tested for the ability to ferment these sugars. Conversion factors were somewhat lower than for LP medium, ranging from 80 to 85% in uninoculated silos to 98 to 100% in treated silos. Raw data were multiplied by the relevant conversion factor as described above.

Chemical analysis of silages. Dry-matter contents of silages and grasses, respectively, were obtained by drying 50 g samples at 40°C for 48 h or at 98°C for 24 h. Water-soluble carbohydrate and pH determinations were carried out on aqueous extracts from grass or silage.

Water-soluble carbohydrates were measured colorimetrically by using the method of Wilson (24). Ammonia nitrogen levels were estimated by using an adaptation of the phenol hypochlorite method of O'Keefe and Sherrington (12).

Crude protein measurements were done on dried, milled forage samples by using a Tecator Kjeltec AUTO 1030 analyzer. Statistical analysis of results from laboratory silos was done by the method of O'Kiely and Wilson (13). Least-squares differences were used to determine significance.

RESULTS

Isolation of *P. acidilactici* strains from silages. *P. acidilactici* isolates were obtained from four grass silage samples from various locations in Ireland as described in Materials and Methods. *P. acidilactici* ferments glucose, fructose, galactose, arabinose, and xylose but not maltose to acid (2), and a single isolate from each sample with such a sugar fermentation profile was selected for further study. Strains A12, B14, and C20 were isolated from well-preserved silages, while G24 was isolated after 2 days of ensilage.

Growth characteristics. The 18 strains of *P. acidilactici* were assessed in the laboratory for their potential as silage inoculants by using a variety of relevant biochemical parameters. All strains were incapable of hydrolyzing gelatin, exhibited no detectable lactate breakdown, and had an optimum growth temperature of 37°C. The 18 strains grew well at temperatures ranging from 25 to 45°C and at pH values between 4.5 and 8. Growth tapered off rapidly at lower values of temperature and pH. Exponential growth rates varied only slightly, ranging from 1.5 to 2.4 h^{-1} on glucose and from 1.3 to 1.9 h^{-1} on fructose. However, significant variation was observed among the strains in their lag phases (5 to 11 h), in the culture pHs after 26 h of growth on glucose and fructose (3.62 to 4.18 and 3.69 to 4.46, respectively), in the glucose-to-lactate conversion efficiencies after 12 h of growth (43 to 78%), in the pH optima for growth (6.0 to 7.3), and in the percentages of maximum growth exhibited at 20°C (12 to 80%). Three strains, *P. acidilactici* PLL03, PLL04, and PLL07, were found to inhibit *L. plantarum* growth. On the basis of these results *P. acidilactici* G24 was selected as having the greatest potential as a silage inoculant by virtue of its short lag phase of 5 h, its ability to produce 80% of the cell mass attained at 37°C at incubation temperatures as low as 20°C, its compatibility with *L. plantarum*, its glucose-to-lactate conversion efficiency of 76% after 12 h, and its ability to reduce the culture pH to 3.62 after 26 h of growth. *P. acidilactici* PLL07 was also tested in laboratory scale silos as a negative control in view of its long lag phase of 8.3 h, its inhibition of *L. plantarum* growth, and its poor ability to reduce the culture pH. Growth curves for these two strains and PLL06 in MRS

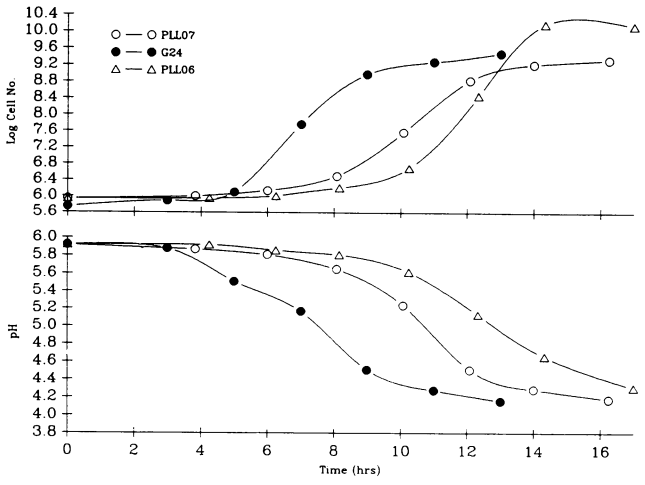

FIG. 1. Growth curves of *P. acidilactici* G24, PLL06, and PLL07 on MRS. The sole carbon source was glucose.

TABLE 1. Treatments used in test silos

Treatment	No. of bacteria added/g of grass	
	Expt 1	Expt 2
1	0	0
2	10^4 *L. plantarum*	10^6 G24
3	10^6 G24	
4	10^6 G24 + 10^4 *L. plantarum*	
5	10^6 PLL07 + 10^4 *L. plantarum*	

with glucose as the sole sugar source are presented in Fig. 1. A similar pattern was observed when fructose was used as the sole sugar source. A good correlation between growth and acidification was observed, with G24, the most active of the strains, rapidly entering the log phase and bringing about the most rapid drop in pH.

Performance in test silos. Two experiments were carried out with the treatments used and the grass compositions outlined in Tables 1 and 2. The changes in the silage microflora and pH over the ensiling period and the silage composition after 6 days are presented in Fig. 2 and 3 and Table 3. Under the good ensiling conditions of experiment 1, inoculation with 10^4 *L. plantarum* cells per g of grass (treatment 2) had no effect on *Pediococcus* numbers, on the rate of lactic acid production and pH decrease, or on the ammonia nitrogen level of the silage after 6 days of fermentation. Inoculation with *P. acidilactici* G24 alone (treatment 3) led to rapid proliferation of pediococci, a 10-fold increase in *L. plantarum* numbers, a significantly greater rate of lactic acid production and pH decrease, and a lower level of ammonia nitrogen than in the uninoculated control. Addition of 10^4 *L. plantarum* cells per g of grass to the G24 inoculum (treatment 4) gave rise to no additional effect on pH, lactic acid production, crude protein, or ammonia nitrogen levels. *P. acidilactici* numbers were unchanged, while *L. plantarum* numbers were significantly lower after 3 days of ensilage than when *L. plantarum* alone was used as an inoculum. Inoculation with *P. acidilactici* PLL07 in conjunction with *L. plantarum* (treatment 5) gave rise to no beneficial effect compared with inoculation with *L. plantarum* alone. *P. acidilactici* numbers were significantly lower than when G24 was used, and *L. plantarum* numbers were unaffected by the presence of strain PLL07.

In experiment 2, only *P. acidilactici* G24, the most successful inoculant from experiment 1, was tested under more challenging ensiling conditions (Table 2 contains a description of the conditions). Figure 3 indicates that inoculation led to a less rapid rise in *Pediococcus* numbers than in experiment 1 and no significant improvement in the rates of lactic acid production and pH decrease or in the final crude protein level compared with the uninoculated control silages was observed. However, the ammonia nitrogen content of the silage after 7 days of fermentation, at 3.9% of total N, was significantly lower than the uninoculated control value of 4.6%.

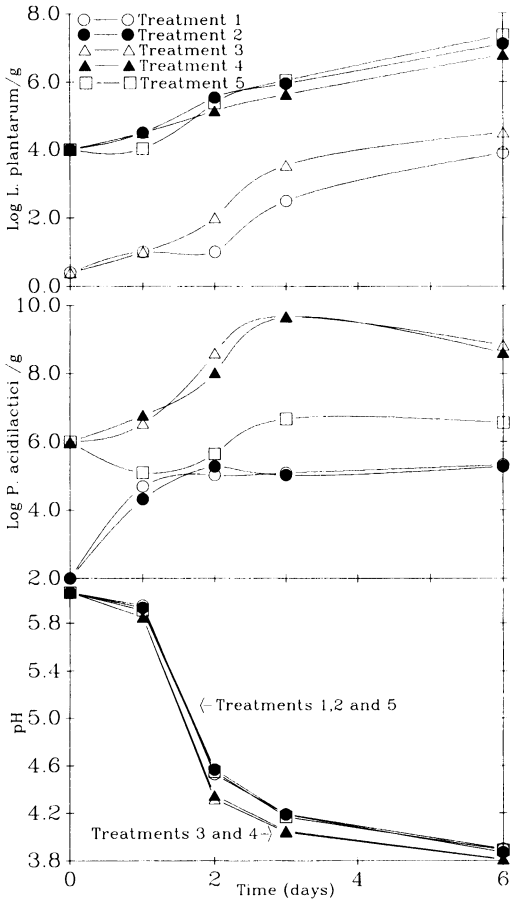

FIG. 2. Changes in silage pH and microbial flora in experiment 1.

DISCUSSION

Of the 18 *P. acidilactici* strains studied, strains isolated from grass silage tended to show common characteristics, distinguishing them from the remaining isolates. Silage isolates A12, B14, C20, and G24 were well adapted to growth at low temperatures, exhibited a short lag phase on glucose and fructose, had a pH optimum for growth of close to 7, and had good acidification efficiency. Since the rapid establishment of a low pH and the domination of the epiphytic microflora are criteria which, above all others, should decide which organisms are used as inoculants (17), *P. acidilactici* G24, which exhibited the shortest lag phase, the most rapid acidification rate, and the fastest growth at 20°C, was se-

TABLE 2. Composition of grasses ensiled

Expt	Mean amt of dry matter (g/kg) (SD)	Mean amt of WSC[a] (g/kg of aqueous extract) (SD)	Mean amt of crude protein (g/kg of dry matter) (SD)	Mean pH (SD)	Mean no. of *L. plantarum* /g	Mean no. of *P. acidilactici* /g
1	154 (2.3)	24 (2.6)	166 (3.6)	6.04 (0.01)	<10	<10
2	135 (2.0)	8 (0.4)	213 (15.6)	6.12 (0.02)	<10	30 (2.5)

[a] WSC, water-soluble carbohydyrate.

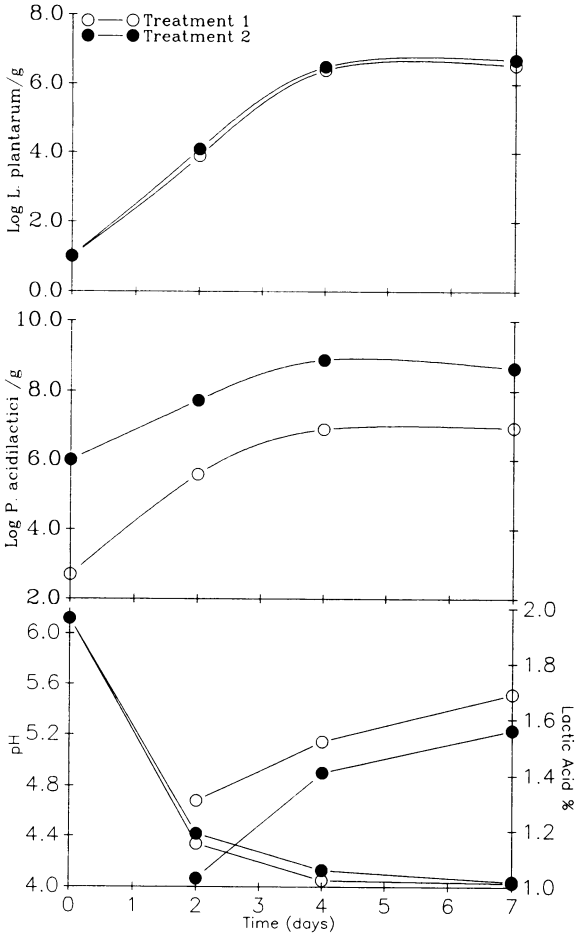

FIG. 3. Changes in silage pH, lactic acid, and microbial flora in experiment 2.

lected for testing in laboratory silos. This strain also fulfilled the remaining criteria for a useful silage inoculant described previously.

The laboratory silo trials were designed in such a manner as to demonstrate whether addition of *P. acidilactici* would affect the silage fermentation, whether any difference could be observed in the performance of *P. acidilactici* strains that differ in their growth characteristics on laboratory growth medium, and to show clearly any synergistic effect between *L. plantarum* and *P. acidilactici*. The inoculation rates of *L. plantarum* were therefore 100-fold less than those of *P. acidilactici* so that any stimulation of *L. plantarum* growth could be easily seen.

In experiment 1, only treatments 3 and 4, consisting, respectively, of *P. acidilactici* G24 alone and in conjunction with *L. plantarum*, were successful, as evidenced by an increased rate of lactic acid production and pH decrease and a final silage with a lower ammonia nitrogen content than the uninoculated control. Addition of 10^4 *L. plantarum* cells per g of grass to the *P. acidilactici* G24 inoculum led to no additional effect on the criteria outlined above. The success of *P. acidilactici* G24 alone as an inoculant is in keeping with the data of Lesins and Schulz (8) and Langston et al. (7), who noted a correlation between high numbers of pediococci in the early stages of ensilage and good preservation. This suggests that a *Pediococcus* sp. capable of dominating the early stages of ensilage would make an effective inoculant. Of the 18 *P. acidilactici* strains studied, G24 is uniquely suited to this task, having been isolated from the early stages of a silage fermentation.

A stimulatory effect of *P. acidilactici* on *L. plantarum* numbers was seen only with treatment 3, in which *P. acidilactici* G24 was used on its own. By day 2, a 10-fold increase in indigenous *L. plantarum* numbers was evident compared with uninoculated controls. Inoculation with a cocktail containing a 10^6:10^4 ratio of *P. acidilactici* and *L. plantarum* per g of grass led to a halving of *L. plantarum* numbers compared with treatment 2, in which *L. plantarum* was used on its own. This suggests that either *P. acidilactici* G24 stimulates the growth of indigenous *L. plantarum* only or that any synergistic effect is dependent on the initial ratio of the two strains in the silo; i.e., when *P. acidilactici* and *L. plantarum* are present in an initial ratio of 10^6:10/g of grass, *P. acidilactici* stimulates *L. plantarum* growth. However, at higher *L. plantarum* levels the two strains compete for water-soluble carbohydrate, with *P. acidilactici* becoming dominant in the trials described here. The microbiological data also indicate that it is in the early stages of silage fermentation that pediococci exert the most influence, with *P. acidilactici* numbers peaking after 3 days of ensilage and *L. plantarum* numbers still rising after 6 days. This is in agreement with previous studies on succession (7, 9, 23).

We therefore propose that a bacterial inoculant containing only an active *P. acidilactici* strain could prove useful as a silage inoculant, with the *Pediococcus* sp. dominating the early stages of ensilage and promoting a rapid pH decrease while stimulating the natural *L. plantarum* population, ensuring that it will dominate in the latter stages. However, care must be taken in the selection of *P. acidilactici* strains for use as silage inoculants, as indicated by the failure of *P. acidilactici* PLL07 to aid preservation in laboratory silos. The contrasting effects of *P. acidilactici* G24 and PLL07 on preservation appear to validate the laboratory selection methods employed here, in particular, the use of growth curves and acidification rates on MRS. Consideration must

TABLE 3. Composition of silages in experiment 1 after 6 days of ensilage[a]

Treatment	pH	% Lactic acid	Log no. of *P. acidilactici*	Log no. of *L. plantarum*	WSC (g/kg of aqueous extract)	Crude protein (g/kg of dry matter)	Ammonia N (% total N)
1	3.90*	1.74*	5.33*	3.91*	1.0*	161*	5.7*
2	3.87*	1.84*	5.28*	7.10†	0.9*	175†‡	5.1†
3	3.81†	2.06†	8.92†	4.56‡	1.0*	179†	4.6‡
4	3.81†	1.92*†	8.60†	6.78§	0.9*	177†	4.6‡
5	3.88*	1.84*	6.56‡	7.35†	0.8†	170‡	5.4*†

[a] Values within rows that differ significantly from one another are followed by different superscripts. The values shown represent means from three silos.
[b] WSC, water-soluble carbohydrate.

also be given to crop characteristics before selecting an additive, as under the more difficult ensiling conditions of experiment 2, *P. acidilactici* G24 failed to produce any beneficial effect. This may have been due to the low level of crop water-soluble carbohydrate, which has frequently been cited as the reason for inoculant failure (17, 18, 25). This might be overcome by addition of a carbohydrate source, e.g., molasses, to the crop.

ACKNOWLEDGMENTS

This work was supported in part by the Commission of the European Communities, ECLAIR programme, AGRE 0041.

We thank Tom Keating, John Marron, Jim Hammill, Stephen O'Connell, and Noel Tonge for assistance.

REFERENCES

1. **Bernfeld, P.** 1955. Amylases, α and β. Methods Enzymol. **1**:149–158.
2. **Buchanan, R. E., and N. E. Gibbons. (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed., p. 513–515 and 576–593. The Williams & Wilkins Co., Baltimore.
3. **Dellaglio, F., and S. Torriani.** 1985. Microbiological variations in silage of lucerne and Italian rye grass with added lactic acid bacteria or formic acid. Microbiologie **3**:273–282.
4. **Fenton, M. P.** 1987. An investigation into the sources of lactic acid bacteria in grass silage. J. Appl. Bacteriol. **62**:181–188.
5. **Henderson, A. R., and P. McDonald.** 1984. The effect of a range of commercial inoculants on the biochemical changes during the ensilage of grass in laboratory studies. Res. Dev. Agric. **1**:171–176.
6. **Henderson, A. R., P. McDonald, and M. K. Woolford.** 1972. Chemical changes and losses during the ensilage of wilted grass treated with formic acid. J. Sci. Food Agric. **23**:1079–1087.
7. **Langston, C. W., C. Bouma, and R. M. Conner.** 1962. Chemical and bacteriological changes in grass silage during the early stages of fermentation. II. Bacteriological changes. J. Dairy Sci. **45**:618–624.
8. **Lesins, K., and F. H. Schulz.** 1968. Some effects of bacterial inoculation on silage making. Can. J. Anim. Sci. **48**:15–25.
9. **Lindgren, S., P. Lingvall, A. Kaspersson, A. De Kartzow, and E. Rydberg.** 1983. Effects of inoculants, grain and formic acid on silage fermentation. Swed. J. Agric. Res. **13**:91–100.
10. **McDonald, P.** 1981. The biochemistry of silage, p. 131. John Wiley & Sons, Chichester, England.
11. **Moran, J.** 1990. Enumeration of lactic acid bacteria on grass and the effects on silage fermentation of added bacteria. M.S. thesis. Dublin City University, Dublin, Ireland.
12. **O'Keefe, M., and J. Sherrington.** 1983. Comparison of 3 methods for the determination of urea in compound feed and silage. Analyst **108**:1374–1379.
13. **O'Kiely, P., and R. K. Wilson.** 1991. Comparison of three silo types used to study in-silo processes. Ir. J. Agric. Res. **30**:53–60.
14. **Pahlow, G., and B. Ruser.** 1988. Einfluss von Ernteaufbereitung auf den Nachweis der epiphytischen Laktobakterien von Futterpflanzen. VDLUFA **28**(Teil II):957–966.
15. **Rooke, J. A.** 1991. Lactic acid bacteria and silage fermentations. J. Chem. Technol. Biotechnol. **51**:560–562.
16. **Schlegel, H. G.** 1985. General microbiology, 6th ed. Cambridge University Press, Cambridge.
17. **Seale, D. R.** 1986. Bacterial inoculants as silage additives. J. Appl. Bacteriol. Symp. (Suppl.) **15**:9S–26S.
18. **Seale, D. R., A. R. Henderson, K. O. Pettersson, and F. J. Lowe.** 1986. The effect of addition of sugar and inoculation with two commercial inoculants on the fermentation of lucerne silage in laboratory silos. Grass Forage Sci. **41**:61–70.
19. **Sharpe, E. M.** 1981. The procaryotes. Springer Verlag, New York.
20. **Teagasc.** 1991. Directory of silage additives. Teagasc, Dublin, Ireland.
21. **Weinberg, Z. G., G. Ashbell, and A. Azrieli.** 1988. The effect of applying lactic bacteria at ensilage on the chemical and microbiological composition of vetch, wheat and alfalfa silages. J. Appl. Bacteriol. **64**:1–7.
22. **Whittenbury, R.** 1961. An investigation of the lactic acid bacteria. Ph.D. thesis. University of Edinburgh, Edinburgh.
23. **Whittenbury, R.** 1968. Microbiology of grass silage. Process Biochem. **3**:27–31.
24. **Wilson, R. K.** 1978. Estimation of water soluble and individual carbohydrates in grass samples. Proc. Euroanalysis, abstr. 3, p. 46.
25. **Woolford, M. K.** 1984. The silage fermentation. Marcel Dekker, Inc., New York.